THE BROAD STREET PUMP REVISITED: RESPONSE OF VOLUNTEERS TO INGESTED CHOLERA VIBRIOS*

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THE Broad Street water pump in London was a source of cholera in 1854 as Snow[1] so carefully demonstrated. Snow's work implicated the important role water played in the spread of cholera. He postulated that contaminated water contained some form of viable contagion. Koch[2] was able to verify this hypothesis 30 years later by identifying vibrios from stools of patients with cholera in Egypt and India. The lack of an animal model has inhibited the acquisition of knowledge dealing with infectivity of vibrios. Carpenter et al.[3] have demonstrated many important facts in the pathogenesis of cholera with their canine model. However, the dog is markedly resistant to cholera infection and requires large numbers of organisms to induce disease.[3] Cholera is an enteric disease unique to man. In an effort, therefore, to evaluate vaccines a human model has been developed. This ability to study the induction of cholera in volunteers has established the postulates of Snow and Koch regarding the infectivity and virulence of *Vibrio cholerae* for man. This presentation will deal with results of studies conducted in volunteers infected with varying doses of classic cholera strains.

MATERIALS AND METHODS

Volunteers in these studies were inmates at the Maryland House of Correction. A well-equipped medical ward has been maintained for 10 years at the prison by the Division of Infectious Diseases of the Uni-

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versity of Maryland School of Medicine. Several other enteric and viral respiratory diseases have been studied in this unit. The willingness and eagerness of volunteers to participate in all of these investigations is greatly appreciated and admired. The general plan of the cholera studies has been approved by the Cholera Advisory Committee of the National Institutes of Health. The Committee for Clinical Investigations at the University of Maryland School of Medicine has approved the protocols utilized in the studies. Each volunteer was informed of the nature of the disease and study plan and given several opportunities to withdraw prior to challenge. Appropriate laboratory studies including electrocardiogram, chest x ray, hematology, and blood chemistries were complementary to the histories and physical examinations in the selection of healthy volunteers.

Two strains of *Vibrio cholerae* were employed: Inaba 569B and Ogawa 395, two classic biotypes. Each had been used in the canine model and generously supplied by Charles C. J. Carpenter. The challenge organism was cultured on Brain Heart Infusion Agar (BHIA) overnight at 37°C. Identity was tested with group and type specific antisera, and 20 to 30 colonies were picked and suspended in BHIA. Preincubated BHIA plates were incubated with the BHI suspension. After 5 to 6 hours of incubation each plate was harvested with 5 ml. sterile buffered to *p*H 7.2 + 0.1 with one part M/15 Sorensen's phosphate buffer to three parts saline. The harvested organisms were centrifuged in the cold at 2,500 RPM for 10 minutes. The pellet was resuspended and washed twice in four times the original volume. The suspension was standardized spectrophotometrically, and appropriate dilutions were made to approximate the organism count required for each challenge. Buffer-saline suspensions of the vibrio were made and the challenge organisms were always contained in 1.0 ml. Each inoculum was not only diluted and plated out for enumeration of the inoculum size but, after challenge of volunteers (usually within two hours), a repeat count of the inoculum was carried out to determine if any dying off had occurred between the laboratory manipulations and the time of challenge at the prison. Only those protocols in which adequate reproducibility of pre- and postcounts occurred were accepted for inclusion in the results presented here.

The challenge was conducted by placing the organisms in approximately 30 ml. of water which was then ingested by the volunteer. In

TABLE I. RESPONSE OF VOLUNTEERS TO VARYING DOSES OF *VIBRIO CHOLERAE:* INABA 569B STRAIN (NO BUFFERING)

Dose	*Carrier*	*Diarrhea*	*Cholera diarrhea*
10^4	0/2	0/2	0/2
10^6	0/4	0/4	0/4
10^7	0/4	0/4	0/4
10^8	0/4	2/4 50%	0/4
10^9	0/2	1/2 50%	0/2
10^{10}	0/1	0/1	0/1
10^{11} (Bouillon)	0/2	1/2 50%	1/2 50%
Totals	0/19	4/19 21%	1/19 5%

Evidence of cholera in 5/19 men: 26%

some studies 2 gm. of sodium bicarbonate in 60 ml. of water was administered just prior to the vibrios. All volunteers were fasted for two hours prior to and after the feeding.

Attempts were made to culture all stools from each volunteer. They were plated directly on Smith's Gelatin Agar (GA) and TCBS media (thiosulfate citrate bile salt sucrose). Subculture was to NGP broth and then into TCBS and MacConkey's agar. All suspect colonies were picked and inoculated into Triple Sugar Iron Agar and tested against antisera by agglutination.

Close clinical appraisal of each volunteer was maintained. Detailed fluid intake and output charts were kept on each individual. When it became apparent that stooling was at a rapid and increasing rate with a lag in oral intake, therapy with intravenous Plasmalyte* was begun. Adequate intravenous fluid was administered to maintain positive balance of fluid. Tetracycline therapy was administered to most of the volunteers who developed cholera diarrhea. In addition all other volunteers received 1 gm. of tetracycline per day for five days prior to discharge from the study.

RESULTS

A wide spectrum of illness occurred after ingestion of varying doses of vibrios. For clarity of presentation of data, the various gradations

*Travenol Co. Contents: sodium 140 mEq./l.; potassium 10 mEq./l.; chloride 103 mEq./l.; acetate 47 mEq./l.; lactate 8 mEq./l.; calcium 5mEq./l.; magnesium 3 mEq./l.

TABLE II. RESPONSE OF VOLUNTEERS TO VARYING DOSES OF *VIBRIO CHOLERAE:* INABA 569B STRAIN (WITH $NaHCO_3$)

Dose	*Carrier*	*Diarrhea*	*Cholera diarrhea*
10^1	0/2	0/2	0/2
10^3	3/4	0/4	0/4
10^4	2/13	9/13 69%	0/13
10^5	1/8	5/8 63%	1/8 13%
10^6	1/23	14/23 61%	6/23 26%
10^8	0/2	1/2 50%	1/2 50%
Totals	7/52 13%	29/52 56%	8/52 15%

Evidence of cholera in 44/52 men: 85%

of infection were classified as follows: *no evidence of infection:* i.e., negative stool cultures and lack of serological response; *carrier state:* the group of volunteers who manifested evidence of infection because of one or more isolations of *Vibrio cholerae* from stool cultures; *diarrhea:* those volunteers with at least one liquid stool containing the etiological organism but never requiring intravenous administration of fluid; and, finally, *cholera diarrhea:* volunteers with severe watery diarrhea requiring IV fluid to maintain fluid balance.

Tables I through IV indicate the frequency of these forms of cholera. The results demonstrate the common occurrence of diarrhea in the challenged volunteers. Some of these men had only a few liquid stools—a condition which would not cause one to seek medical attention. Total stool output measurements in this group ranged from 150 cc. to 42.9 l. Most of the men had 4 to 6 l. total volume of vibrio positive liquid stool. Figure 1 is an example of this mild form of cholera. Usually the diarrhea lasted seven days if untreated with tetracycline.

None of the volunteers with cholera diarrhea became severely dehydrated since intravenous fluid therapy was begun promptly when oral intake failed to balance output. A typical example of induced cholera diarrhea is shown in Figure 2. Note the early minimal diarrhea which began about 24 hours after challenge. Accelerated liquid stooling became apparent after 2½ days and reached a maximum rate on the third day. Tetracycline therapy and intravenous fluid was begun at this point and the rate of almost 1,200 cc. of stool per hour fell quickly, so that

TABLE III. EFFECT OF STOMACH BUFFERING ON INCIDENCE OF CHOLERA INFECTION

Strain	*Bicarbonate*	*Dose*	*Diarrhea*	*Cholera diarrhea*
Inaba	0	$10^8 >$	4/8 50%	1/8 13%
Inaba	+	$10^4 >$	29/46 63%	8/16 17%
Inaba	0*	10^3	2/9 22%	0/9

*Intraduodenal challenge

by 48 hours the rate was less than 100 cc. and by three days the diarrhea had ceased. The total stool volume in this man was 42.5 l. over a seven-day period.

Incubation periods on the mild diarrhea and cholera diarrhea was 47 to 36 hours respectively. In about 70% of the cases these initial stools yielded *Vibrio cholerae;* however, formed stools culturally positive for the vibrios were noted in 19% of the volunteers and, conversely, 12% of volunteers had liquid stools which failed to demonstrate vibrios upon culture.

Cholera in the volunteers had incubation periods which varied inversely with dose—the larger the dose, the shorter the incubation period.

Table I summarizes the experience with strain 569B in volunteers when administered without any buffering. These are washed organisms in pure culture (in the hope of reducing the amount of preformed toxin ingested). Nineteen men received from 10^4 to 10^{10} organisms. No evidence of altered stool characteristics or isolation of vibrios occurred until a dose of 10^8 organisms was swallowed. The two men who were given 10^{11} organisms had simultaneous beef bouillon in order to give a medium in which the vibrios could survive in the gastrointestinal tract. In these 19 men only 26% demonstrated some evidence of cholera, which indicated that healthy American males were not exquisitely sensitive to cholera. Many reasons for this apparent lack of susceptibility exist. A few will be mentioned here. First the strain of Inaba 569B was a laboratory-adapted strain that had been isolated in 1964 and that had many dog and rabbit passages prior to introduction to man. To obviate a possible loss of human virulence the organisms isolated from the volunteer who developed cholera diarrhea was utilized as a source of vibrios

TABLE IV. RESPONSE OF VOLUNTEERS TO VARYING DOSES OF *VIBRIO CHOLERAE:* OGAWA 395 STRAIN WITH ($NaHCO_3$)

Dose	*Carrier*	*Diarrhea*	*Cholera diarrhea*
10^3	0/2	1/2	0/2
10^6	0/22	11/22	9/22
	0/24	12/24 50%	9/24 38%

Evidence of cholera in 21/24 men: 88%

for subsequent studies. No obvious enhancement of virulence with this human isolate was noted.

Other factors involved in this host-parasite interaction include specific and nonspecific defense mechanisms. Cholera is not an endemic disease in this country and it would seem unlikely our volunteers would have the opportunity to develop immunity to cholera. They would be lacking in protective antibodies. Serum antibody data will be presented later but no evidence existed to suggest these men had significant humoral antibodies.

Nonspecific immune mechanisms are poorly understood. Acidity of the stomach has long been considered the first line of defense against many enteric infections, especially cholera. Table II demonstrates the enhancement of the virulence of vibrios by the addition of a buffering agent to the inoculum. Note that 10^4 organisms was sufficient to induce diarrhea and that active cholera occurred after 100,000 vibrios. Increasing the dose of organisms produced a greater number of volunteers with cholera diarrhea while the proportion of mild cases of diarrhea remained the same. Over-all evidence of cholera was detected in 85% of the volunteers. Thus only 15% of those men ingesting bicarbonate and organisms were able to eliminate the ingested vibrios. However, it is of interest that even after 1,000,000 organisms about 9% of volunteers had no evidence of infection. Temporary carrier states occurred in 13%. Asymptomatic individuals such as these could be a source of further cases of cholera.

Table III compares the attack rates of cholera in volunteers infected with Inaba 569B with and without bicarbonate or directly into the duodenum. The addition of bicarbonate lowered the number of organ-

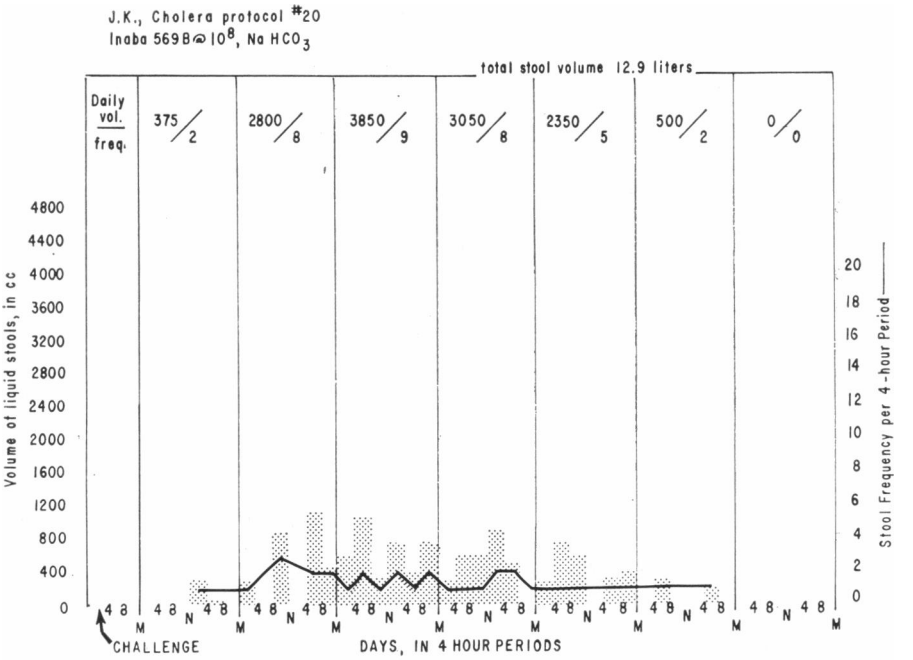

Fig. 1. Mild diarrhea.

isms required to cause disease from 10^8 to 10^4 organisms. These data suggested that the stomach was an effective barrier to further infestation by vibrios. Placing the organisms beyond the stomach did not result in any clear-cut conclusion but in at least two of these nine men the *p*H of the duodenal contents was acid and in one was alkaline. Perhaps the acid effect was not completely bypassed by introducing the tube from the stomach into the duodenum. The persisting acidity in the duodenum would be capable of inactivating the organisms.

Table IV outlines the virulence of Ogawa 395 in man. There was a suggestion that this strain caused more severe disease in the volunteers than Inaba; however, additional evidence is needed to confirm this trend.

The mechanism (or mechanisms) involved in the synergistic effect of bicarbonate was investigated in volunteers. Estimation of the *p*H of gastric contents was chosen as the simplest parameter to measure in man. The day prior to challenge a nasogastric tube was placed in the

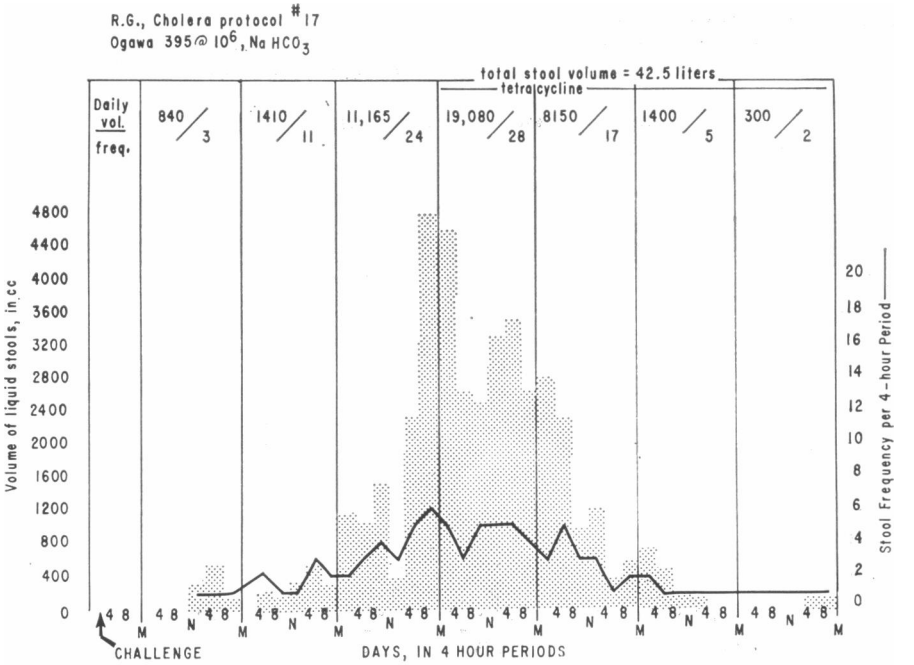

Fig. 2. Cholera diarrhea.

fasting volunteer. Stomach contents were aspirated every 15 minutes until 5 samples were collected. Two grams $NaHCO_3$ in 50 to 60 ml. of distilled water were drunk and four more samples were collected at 15-minute intervals. The *p*H of each sample was determined with a Beckman *p*H meter. In 36 men the contents of the stomach were acid prior to bicarbonate with the usual *p*H less than 2.0. Analysis at 15-minute intervals of the *p*H change revealed two distinct groups. One: the stomach contents were buffered to *p*H 5.0 or greater; two: acid secretion in the second group rapidly overcame the buffering effect and the *p*H returned to basal acid levels. The pharmacological effect of bicarbonate on gastric acid is a dynamic process. Immediately after the ingestion of bicarbonate probably all the men had a significant buffer effect. The duration of this presumed buffering action was less than 15 minutes in nine men. At 30 minutes 50% of this group had lost the measured buffering effect. By 45 minutes approximately 80% had returned to basal acid level, and no significant buffer effect was found at 60 minutes.

TABLE V. DOSE-SPECIFIC ATTACK RATES IN SECRETOR AND NONSECRETOR GROUPS

	15 minutes after $NaHCO_3$		*30 minutes after $NaHCO_3$*	
	Secretor	*Nonsecretor*	*Secretor*	*Nonsecretor*
10^4	1/4 = 25%	4/5 = 80%	1/5 = 20%	4/4 = 100%
10^6	2/4 = 50%	12/13 = 92%	5/7 = 71%	9/10 = 90%
10^8	1/1 = 100%	1/1 = 100%	2/2 = 100%	—
	4/9 = 44%	17/19 = 90%	8/14 = 64%	13/14 = 93%

Cholera vibrios are exquisitely sensitive to low *p*H. The 15- and 30-minute postbicarbonate *p*H determinations of the volunteers were correlated with the attack rates of disease. The group of men characterized as overcoming the bicarbonate effect was termed "secretors" and those who remained buffered (*p*H 5.0 or greater) "nonsecretors." These terms have no connotation other than that they are defined relative to each other. The importance of this differentiation in terms of susceptibility to cholera is presented in Table V.

Though the numbers are small, attack in the secretor groups were less than in those individuals with a prolonged buffering effect. The increasing attack rate with increasing vibrio dose in the secretor group was also a suggestive trend. That the same vibrio doses induced disease in almost all the nonsecretors implied that low gastric *p*H was an important defense against induced cholera.

Serological studies indicated that those men with the most severe disease had demonstrable agglutinins and vibriocidal antibody titers in their serum specimens. The volunteers with temporary carrier states failed to develop humoral antibodies. The development of cholera diarrhea was associated with significant increases in titers of vibriocidal and agglutinating antibodies. However, attempts to correlate baseline vibriocidal antibody titers and immunity have failed to give clear-cut differentiation. There was a trend of greater susceptibility to infection when no vibriocidal antibody titer was present. The significance of these vibriocidal antibodies in a population group unexposed to cholera raises some doubt as to their specificity. Perhaps they were a measure of some cross-reacting antibody and therefore have less relevance than vibriocidal antibodies measured in natives of endemic areas. Additional information will be accumulated on this point.

Rechallenge studies have demonstrated a remarkable resistance to a second dose of organisms. Thus homologous strain rechallenge of 13 volunteers recovered from diarrhea or cholera three to 12 months previously resulted in complete immunity. No stool cultures were found to have vibrios. Heterologous strain rechallenge failed to demonstrate complete immunity. Thus two individuals manifesting diarrhea after an Inaba challenge had a similar illness following Ogawa challenge six to nine months later. Two other volunteers developed diarrhea and carrier status on rechallenge whereas the initial challenge caused only carrier states. These data do suggest a possible role for oral living vaccines as a means of preventing cholera.

Discussion

Dr. John Snow suspected that a living contagion was contained in the foul water pumped into the Broad Street area. His careful studies pinpointed water as a means of spreading cholera. The diagnosis of cholera in his era could be made only on a clinical basis in the severely dehydrated, usually fatal cases. His analysis compared fatal cases per 10,000 population to the source of their water supply. The actual rate of cholera infection was probably 10 or more times the fatal case rate. The majority of exposed individuals develop mild self-limiting diarrhea and a small percentage have an occult intestinal infection. Such individuals can promulgate the disease by their carrier state. Susceptibility to infection is modified by many factors, one of which is the status of the acid milieu of the stomach. We have little knowledge of the incidence of achlorhydria or hypochlorhydria in the 1850's in England. Snow did notice an increased rate of disease in the poor who lived in crowded homes, and he implied that these individuals may have had greater contact with the causative agent. It is interesting to speculate that perhaps these individuals did indeed have poor acid secretion in the stomach and were therefore more susceptible. In recent epidemics it has been noted that an inordinate number of patients developing cholera have had previous gastrectomies. Pierce[4] has recently demonstrated altered gastric acid secretion in patients during acute cholera. This suggests either that the disease has an inhibiting effect on gastric acid secretion or that the disease had selected out those individuals deficient in acid production.

The vivid descriptions in Snow's writings as to the turbidity of

water samples from the Broad Street pump and other water sources in London point to the probability that many bacteria were in suspension. The inoculum from the Broad Street pump might well have been 10^6 or more organisms per cubic centimeter of water. Such a large dose would insure disease even in patients with a normal stomach. Unfortunately Snow was not able to culture the water to isolate vibrios in 1854. If such an experiment could have been done he could have supplied us with significant data on the number of organisms ingested in nature that cause disease in man. It remains to be demonstrated as to whether coliform bacteria in contaminated water can act synergistically with vibrios in enhancing disease. Under these circumstances perhaps less than 100,000 organisms could initiate disease.

Specific measures to control cholera such as effective vaccines are still in preliminary testing phases. Snow's removal of the pump handle appeared to abort the epidemic in 1854. Unfortunately a similar simple means of interrupting the present spread of cholera does not exist. Until we can provide clean water supplies or an effective vaccine to all individuals this interesting disease will persist.

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